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RESEARCH PAPER

In vitro bioassay to study phytotoxicity affects of *Quercus leucotrichophora* on some small millets varieties

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The present study was conducted for the evolution of phytoxiticy of leaves and bark extracts of *Quercus leucotrichophora* on seed germination and radical and plume growth of different small millets verities. On average leaves extracts were more toxic to germination and growth of test crops as compared to barks extracts. Lower concentration of leaves and barks extracts stimulated the germination and radicle and plumule growth of the test crops as compared with the control. These results also indicated that radicle growth of test crops were more inhibited as compared to the plumule growth in all the text crops, irrespective of concentration. On an average, germination of all the small millets varieties resistance against the leaves and barks extracts. While seedling growth of *Echinochloa frumentacea and Amaranthus caudatus* showed some resistance and *Triticum aestivum* and *Eleusine coracana* were sensitive crops.

Key words : Allelopathy, Germination, Radicle, Plumule, Small millets

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INTRODUCTION

Agroforestry refers to practices, which deliberately or intentionally mix or retain woody perennials on the crop/animal production systems. It combines elements of agriculture, crops and/ or animals with elements of forestry in production system in a unit of land, either simultaneously or sequentially. The term woody perennial includes tree, shrubs, bushes, palm, bamboos, etc. which in agroforestry context are often referred to as multipurpose trees and shrubs (Wood, 1988). The word deliberate is also significant- a few trees remaining during the process of land clearance for agriculture is not agroforestry. To qualify as agroforestry, a system should actively promote the woody perennials for a particular purpose, or purposes, on the farm (Bhatt and Verma, 2002). Allelopathy as a natural phenomenon in plant–plant interaction plays an important role in agro-ecosystems. Rice (1984) defined the allelopathy phenomenon as process by which plants release chemical compounds in their environment, to keep themselves with a competitive advantage (Kong *et al*., 2004). Agroforestry trees of home gardens are rich sources of secondary metabolites (allelochemicals). The accumulation of tree part *i.e.* leaf, small twigs, bark and fruits on soil in agroforestry systems have deleterious effects on the germination and growth of crops (Rice, 1984). Many studies have been carried out on influence of agroforestry tree species on associated crops in Garhwal Himalaya (Todaria *et al*., 2005; Singh *et al*., 2008; Singh *et al.*, 2009; Todaria *et al*., 2001 and Singh *et al*., 2010).

Quercus leucotrichophora A.Camus. ex. Bahadur belong to family Fagaceae is a moderate-sized/large evergreen tree attaining a height of 12-18m (Gaur, 1999). *Q. leucotrichophora* is a commonest oak of the western Himalaya, extending eastward to Nepal, chiefly in the outer ranges from 1,000- 2,400m, but occasionally descending lower in moist situations (Negi and Naithani, 1995). Now-a-day's advancement in the research field has been made to study the allelopathic interactions between crops and weeds, crops and crops, crops and trees both stimulatory and inhibitory (El-Darier, 2002; Hemada *et al.*, 2004; Abou-Zeid and EL-Darier, 2014). Keeping in view of above mentioned facts, present study was carried out to investigate the *in-vitro* phytoxicity of *Q. leucotrichophora* promising agroforestry tree species on small millets verities of Garhwal Himalaya, India.

RESEARCH METHODOLOGY

The experiments were conducted in the Department of forestry, Ranichauri, Tehri Garhwal (Uttarakhand). To examine the phytoxicity of leaves and bark of *Quercus leucotrichophora* were collected from nearby campus from the selected trees. Four small millets verities *i.e. Echinochloa frumentacea* (PRJ 1) and *Amaranthus caudatus* (PRA 1) and *Triticum aestivum* (UP-1109) and *Eleusine coracana* (PRM 1) were collected from crop improvement, Department of VCSG UUHF Ranichauri.

The collected fresh plant parts were cleaned with tap water to remove soil and dust and dried in shade under laboratory conditions. The dried plant material were chopped into small pieces with sharp knife and further dried in oven at 50^0C for 48 h and ground in electrical grinder.

Lab bioassay :

A sample of 40 g of bark and leaves from each species was added to 400 ml of double distilled water separately and left for 24 hr. at room temperature. The resulting brownish and dark extract were filtered through three layers of Whatman number 1 filter paper and stored in conical flasks. Aqueous extract was further diluted to 25 per cent, 50 per cent, 75 per cent and 100 per cent concentration. The resulted extracts were stored at refegerator. The effect of extracts on seed germination, radicle and plumule growth of different small millets varieties was tested by placing 100 seeds (four replicates of 25 seeds each) of each test crop in Petri dishes containing three layers of Whatman number 1 filter paper saturated with particular extract at room temperature. A separate control series was set up using distilled water. Moisture in the Petri dishes was maintained by adding extract or distilled water as required. The number of seeds germinated was counted daily for 7 days after which the observations were stopped. It was deemed right to stop measurements after seven days since radicle in Petri dishes normally started shriveling at their tips and any further reading, thereafter, could introduce errors to the data. Germination percentage was computed separately and data were analyzed statistically.

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Germination :

The effect of *Quercus leucotrichophora* leaves and bark pulp extracts were studied on germination of test crops (Table 1). Leaves extracts (100.0%) showed maximum (66.0 %) reduction in germination of *Amaranthus caudatus*. On the other hand it was interesting to see that lower leaves and bark extract

Table 1: Effects of leaves and bark extract of *Quercus leucotrichophora* on seed germination of test crops

Treatments	*Echinochloa frumentacea*		*Amaranthus caudatus*		*Triticum aestivum*		*Eleusine coracana*	
	Leaves	Bark	Leaves	Bark	Leaves	Bark	Leaves	Bark
Control	92±1.9	95±2.2	95±2.1	97±1.0	97±0.7	98±1.4	57±3.0	63±3.2
10 %	100±0.0	98±2.0	82±4.3	100±0.0	100±0.0	100±0.0	67±2.2	65±2.2
20%	94±3.2	98±2.1	50±3.5	98±1.6	97±3.0	97±2.2	52±2.9	62±2.3
50%	94±3.7	97±2.2	40±3.8	98±2.4	94±2.4	95±2.2	48±1.4	58±3.4
75%	90±4.4	95±3.3	32±4.5	97±2.7	91±2.2	93±3.1	42±1.9	53±2.5
100%	85±3.3	95±3.5	32±2.5	84±3.2	88±3.7	85±3.1	35±1.4	52±3.7

stimulated germination in all the test crops except leaves extracts on *A. caudatus* as compared to control.

Radicle and plumule growth :

Table 2 depicts the data, phytoxicity of *Q. leucotrichophora* on radicle and plumule growth of small millets as a test crops. Leaves extracts (100 %) of *Q. leucotrichophora* exhibited maximum (40.6 and 53.3 %) reduction in radicle and plumule growth of *Triticum aestivum* and *A. caudatus,* respectively. Bark extracts (100 %) proved maximum (26.86 and 16.17 %) reduction in radicle and plumule growth of *T. aestivum*. While lower 10 per cent concentration of bark extracts stimuled radicle and plumule growth of all test crops except *Eleusine coracana*.

Preliminary bioassay study was conducted to investigate the influence of *Q. leucotrichophora* on germination and growth of important field crops and the results revealed its significance to introduction in existing agroforestry system of Garhwal Himalaya. But the present study was conducted on the estimate the phytoxiticy of *Q. leucotrichophora* on some improved varieties of small millets. The results of present study showed inhibitory or stimulatory effect of various extracts concentration of *Q. leucotrichophora* on germination and growth response of the small millets. Among these, leaves extracts showed greater reductive effect on *A. caudatus,* however, *E. coracana* was moderately resistance while *Echinochloa. frumentaceae* and *T. aestivum* were slightly affected under various concentrations of extracts.

Most published work has revealed that foliage extracts are produced more toxic metabolites and their toxic effects are species specific (May Fiona and Ash, 1990; Bhatt and Todaria, 1990 and Todaria *et al*., 2010). A large diverse allelochemicals are leach out with organic and inorganic compounds such as phenolic acids, terpenoides and alkaloids (Tukey, 1970). Differential inhibitory effects of various parts of the same plant are likely due to variability in the amount of phytotoxic compounds in different plant tissues (Rice, 1974; Nishmura *et al.,* 1982 and May Fiona and Ash, 1990).

The stimulatory response of lower concentration of leaves and bark extract of *Q. leucotrichophora* on germination and growth extension of investigated small millets crops may be due to presence of less tannin contents while, reduction in germination and growth under treatments of different higher concentration of leaves and bark extracts may be due to the presence of higher amount of tannin and phenolic contents (Anonymous, 1976). Tanveer *et al*. (2010) also found the effect of *Euphorbia helioscopia* aqueous extract on *Triticum aestivum*. Singh *et al*. (2012) also revealed that the leaf leachates of *Paulowania* and *Poplar* species significantly affected germination of maize and wheat. *Salix alba* leaf aqueous extracts significantly inhibited germination percentage, plumule

Table 2: Effects of leaves and bark extract of *Quercus leucotrichophora* on radicle and plumule growth of test crops

Treatments	*Echinochloa frumentacea*		*Amaranthus caudatus*		*Triticum aestivum*		*Eleusine coracana*	
	Radicle	Plumule	Radicle	Plumule	Radicle	Plumule	Radicle	Plumule
Leaves extract								
Control	5.4±0.4	6.9±0.9	6.4±0.8	4.1±0.2	7.1±0.6	9.2±0.5	3.0±0.5	3.6±0.1
10 %	6.3±0.7	9.2±0.6	6.6±0.4	5.2±0.6	7.9±0.7	8.9±0.3	2.3±0.3	3.1±0.4
20%	6.0±0.9	8.9±0.6	5.1±0.9	5.3±0.3	7.4±0.5	8.3±0.1	2.8±0.1	3.0±0.3
50%	5.0±0.5	8.3±0.8	5.6±0.6	3.6±0.6	5.3±0.2	6.9±0.2	2.1±0.2	3.2±0.5
75%	4.8±0.6	7.8±0.1	4.1±0.3	3.6±0.7	5.5±0.4	5.8±0.4	2.4±0.4	2.9±0.2
100%	3.1±0.3	5.2±0.5	3.8±0.2	1.7±0.4	3.4±0.6	4.3±0.8	2.1±0.8	3.1±0.2
Bark extract								
Control	6.1±0.3	9.7±0.7	6.1±0.8	4.6±0.1	6.7±0.7	10.7±0.7	3.0±0.3	3.7±0.3
10 %	6.7±0.5	10.0±0.8	6.4±0.2	5.5±0.2	7.0±0.6	11.5±0.6	3.0±0.1	3.6±0.3
20%	6.4±0.1	10.2±0.4	7.6±0.7	5.1±0.9	7.3±0.6	11.2±0.6	2.8±0.5	3.0±0.1
50%	6.3±0.2	8.5±0.8	7.2±0.3	4.3±0.2	5.9±0.9	11.3±0.8	2.7±0.6	3.3±0.1
75%	6.1±0.7	6.4±0.6	6.9±0.3	4.5±0.5	5.7±0.9	9.2±0.9	2.5±0.3	3.6±0.6
100%	5.4±0.2	6.2±0.6	6.8±0.8	3.5±0.6	4.9±0.4	7.9±0.8	2.4±0.1	3.4±0.2

and radicle lengths, as well as dry weight of seedlings of four *Triticum aestivum* cultivars and one *Triticum durum* cultivar (Latif *et al.*,2015). They also observed that the effect of leaf aqueous extracts was significant. Mishra (2015) reported the inhibited allelopathic effect of *Lantana* on germination, growth and metabolism of crops, weeds and vegetables crops.

On the basis of our results, leaf extracts was found toxic to germination and growth for different test crops but their degree of toxicity could not higher in these improved varieties, as compared the earlier study conducted by Bhatt and Chauhan (2000). Therefore, *Q. leucotrichophora* is a promising agroforestry tree species which can play an important role in the conservation of ecological balance with upliftment of socio-economic conditions of hill farmers.

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